Toxicity Testing Spokane River Sediments

Quality Assurance Project Plan

by
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Washington State Department of Ecology
Environmental Assessment Program
Watershed Ecology Section

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Project Description

The Washington State Department of Ecology (Ecology) Toxics Cleanup Program (TCP) wants to assess the toxicity potential of sediments in the Spokane River, known to be contaminated with metals, and, in some areas, PCBs and polyaromatic hydrocarbons (PAH). Sediment bioassays have been conducted previously, but these have largely been limited to the Spokane River Arm of Lake Roosevelt, one of the least contaminated parts of the river (Bortelson et al., 1994). In 1994, Ecology bioassayed one sample each from the Spokane Arm, Long Lake, and near Upriver Dam, with results showing some evidence of toxicity (Batts and Johnson, 1994).

TCP requested that the Environmental Assessment Program (EAP) review existing sediment data on the Spokane River to evaluate selected known and suspected contaminated locations. Sediment samples are to be collected from these areas and subjected to the bioassays and chemical analyses listed below, selected by TCP. The objective is to apply bioassays as part of a multiple lines of evidence investigation of sediment toxicity.

Bioassays
Microtox® 100 % Porewater (Acute)
Hyalella azteca 28-day Survival & Growth (Chronic)
Chironomus tentans 20-day Survival & Growth (Chronic)

Chemistry
Priority Pollutant Metals
PCBs
Semivolatile Organic Compounds
Grain Size
Total Organic Carbon

Selection of Sampling Sites

Data on the concentrations of metals, PCBs, and semivolatiles in Spokane River sediment are reported in Bortelson et al. (1994), Batts and Johnson (1994), Johnson et al. (1994), Serdar et al. (1994), EILS (1995), Horowitz (unpublished), and Johnson (2000). Figure 1 shows the locations that have had the ten highest concentrations of zinc, cadmium, lead, total PCBs, or total PAH. Concentrations of other metals and semivolatile compounds are generally low in Spokane River sediments. Pesticides have also not been detected in significant concentrations in the Spokane (Davis et al., 1995; Davis 1998; Serdar et al., 1994).

Figure 1 represents results from all the data collected since 1993, except as follows. A series of USGS samples between Myrtle Point (river mile 84.6) and the state line,

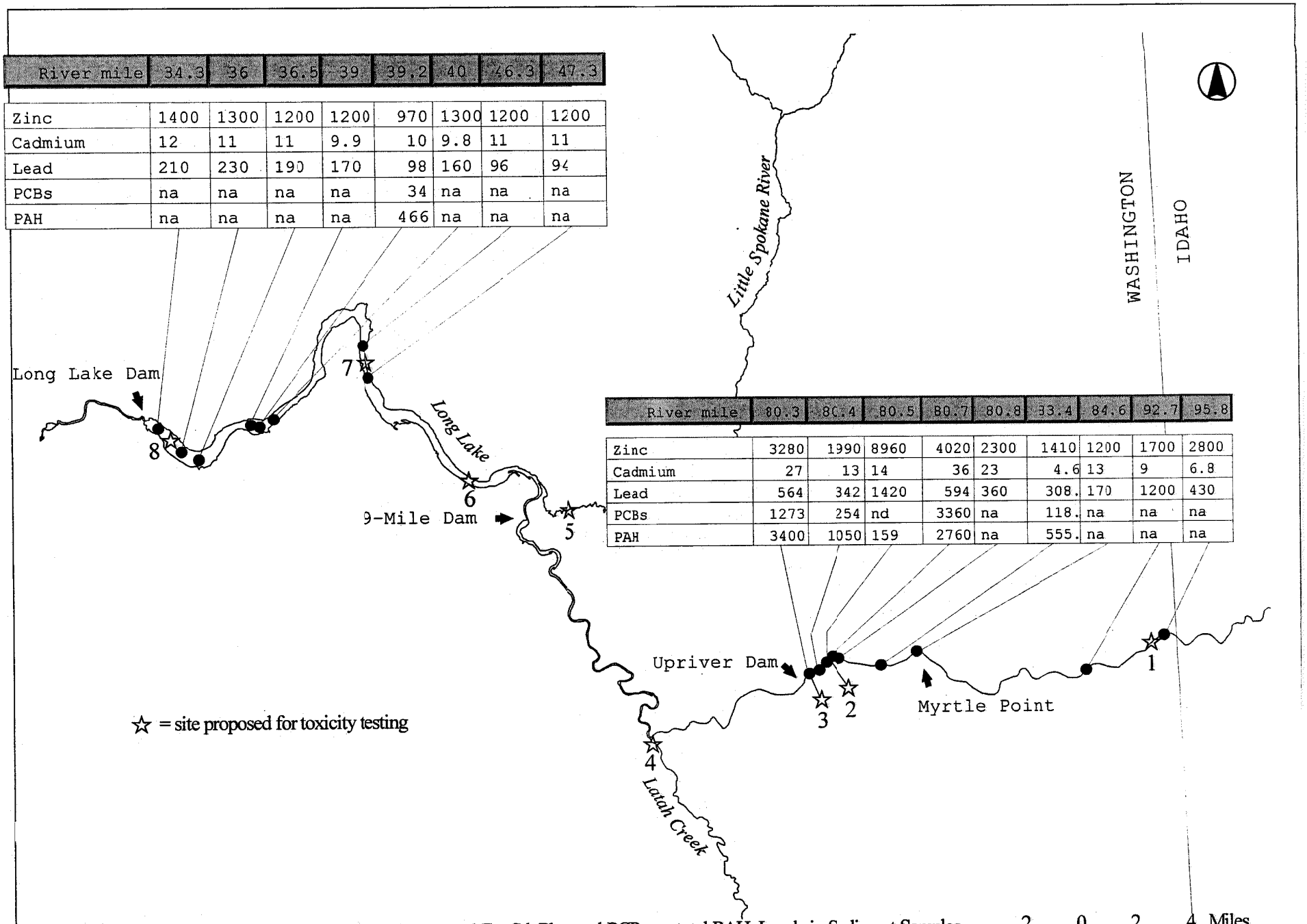

Figure 1. Spokane River Locations With Highest* Reported Zn, Cd, Pb, total PCBs, or total PAH Levels in Sediment Samples (metals in mg/Kg; PCBs and PAH in ug/Kg; dry weight basis)
*see text

sampled by Horowitz in 1998, were not included because these were taken from very small, scattered deposits of material. The zinc, cadmium, and lead concentrations in some of these samples were in the same range as the upper river samples plotted in Figure 1. Also excluded for the same reason were three samples from isolated pockets of material between Upriver Dam and Latah Creek that were somewhat elevated in total PCBs (31 – 390 ug/Kg) (EILS, 1995).

As shown in Figure 1, the most contaminated sediments have been found immediately above Upriver Dam (river mile 80.2). While metals concentrations continue high up to and beyond the state line, PCB concentrations above Mrytle Point (river mile 84.6) are generally low, major historical sources being located upstream. PAH analysis in the reach above Upriver Dam has been limited to sediments below Mrytle Point, with elevated concentrations being found at two sites near the dam.

The reach between Latah Creek and Nine-Mile Dam has not been sampled extensively, but appears to have relatively low levels of contamination. Downstream of Nine-Mile, the middle and lower parts of Long Lake have zinc and cadmium concentrations comparable to sediments in the upper river. PCBs and PAH have only been analyzed at two locations in Long Lake (river miles 39.2 and 50.0) and concentrations have been moderate to low.

Based on this information, the nine sites shown in Table 1 are proposed for toxicity testing:

Table 1. Sediment Sampling Sites Proposed for Spokane River

Site No.	Location	Spokane River Mile
1	Near Stateline	92.7 -95.8
2	0.5 Mi. above Upriver Dam	80.7
3	0.1 Mi. above Upriver Dam	80.3
4	Latah Creek	72.2
5	Little Spokane River	55.3
6	Upper Long Lake	53.6
7	Middle Long Lake	46.8
8	Lower Long Lake	35.2
9	Bead Lake (reference)	- -

Site 1 near the state line was selected as being at the upstream end of the study area and having high concentrations of zinc, cadmium, and lead in the fine sand-sized and less fractions. The location and practicality of sampling in this area is uncertain. The deposits at river mile 92.7 and 95.8, sampled in 1998, were of limited extent and would be hard to re-locate. The Eastern Regional Office (ERO) TCP has suggested having the bioassay sample collected at river mile 95 where EPA soil samples from the Star Road/Island Bar complex averaged 1,410 ug/Kg lead (EPA, 2000b). If aquatic deposits of fine material cannot been found off the bar, ERO TCP doubts if an appropriate area in the upper river can be located for sampling, based on their recent experience.

Sites 2 and 3 above Upriver Dam are in the area most highly contaminated with metals, PCBs, and PAH. Although relatively close together, two sites are proposed because the sediments here are heterogeneous, both chemically and physically (Johnson, 2000).

ERO TCP has requested samples be taken at the mouth of Latah Creek (Site 4), a major source of sediment to the reach behind Nine-Mile Dam, an area not being sampled during the present survey. Ecology and USGS have analyzed metals in two sediment samples collected at the mouth of Latah Creek and found low concentrations of zinc (45 - 64 mg/Kg), cadmium (<0.1 - <0.5 mg/Kg), and lead (8 - 33 mg/Kg) (Johnson, 1999; Horowitz, unpublished). Organic compounds have not been analyzed.

A reference sediment with natural physical and chemical characteristics similar to the Spokane sediments being tested is needed for the bioassays. ERO TCP proposed Site 5, the Little Spokane River near Aubrey L. White Park and Site 9, Bead Lake in Pend Oreille county, 12 miles NE of Newport.

Ecology and USGS have analyzed metals in two sediment samples collected at the mouth of the Little Spokane and found low concentrations of zinc (60 – 97 mg/Kg), cadmium (<0.1 – 0.1 mg/Kg), and lead (10 – 24 mg/Kg) (Johnson, 1999; Horowitz, unpublished). Organic compounds have not been analyzed.

Bead is a 200 acre lake largely surrounded U.S. Forest Service (USFS) land. USFS has analyzed lead and PAH in the lake sediments. The levels are reported to be very low, but the data are not presently available (Bert Wasson, USFS Colville, personal communication).

Sediment samples will be collected from both the Little Spokane and Bead Lake. Bead Lake will be used for the reference sample in the bioassays because of its perceived lower potential for toxicity. Depending on the outcome of the laboratory analyses, the Little Spokane may prove to be a suitable reference for future studies.

Finally, three sites are proposed for Long Lake. Site 8 near Long Lake Dam has the highest zinc, cadmium, and lead concentrations previously detected in the lake. Relatively high levels of zinc, cadmium, and lead extend upstream to the middle part of

the lake at Site 7. ERO TCP has requested that a third site (Site 6) be sampled in the upper Lake. This sample will be collected near Horowitz' site SRG-8 which had the highest metals concentrations among the several sites sampled in the upper lake.

Schedule

October 23 - 25, 2000.....Sediment sample collection
October 26, 2000.....Samples arrive at Manchester Laboratory
December 22, 2000.....Analyses completed and results reported to project lead
January 2001.....Data reviewed and forwarded to clients
March 2001.....Draft project report to clients
May 2001.....Final report completed
June 2001.....Data entered into EIM and SEDQUAL

Project Organization

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TCP Clients - Brett Betts (360/407-6914) John Roland (509/625-5182)
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Jim Laughlin, Parametrix (425/822-8880)

Data Quality Objectives

Precision and Accuracy

PSEP procedures (EPA, 1996) for collection, preservation, transportation, and storage of sediment samples will be followed in an effort to limit sources of bias.

Due to the unknown variables that affect organism response, overlying water quality, and the experience of laboratory personnel, quantitative determination of precision and accuracy in sediment testing of aquatic organisms is difficult (EPA, 2000a). Since there is no acceptable reference material suitable for determining the accuracy of sediment tests, the accuracy of these test methods has not been determined (EPA, 2000a). It therefore becomes important that the testing protocols are followed closely.

The precision Manchester Laboratory routinely achieves with the methods described in this QAPP for physical/chemical analysis of the sediment samples will be acceptable for the purposes of this project. Matrix spikes may provide an indication of bias due to interference from the sample matrix. Surrogate (organics) and laboratory control sample (metals and TOC) recoveries will provide an estimate of accuracy for the entire analytical procedure. Overall precision of the chemical data will be estimated from the results of duplicate analyses and matrix spike/matrix spike duplicates.

Representativeness

Composite samples are being collected in an effort to obtain data representative of each sampling site.

Completeness

The amount of useable data obtained will be maximized by careful planning of field work, packaging, and transport of samples. Excess sample will be collected and held at 4° C in the event the bioassay samples are lost or need to be re-run. For metals and stable compounds like PCBs, holding time for sediment bioassays can be 8 weeks or more at 4° C (EPA, 2000a). Manchester Laboratory will save excess sample for 60 days from the time the data is sent to the project lead to give time for its review.

Comparability

The *Hyalella* 10-day acute bioassay is routinely employed in freshwater sediment studies throughout the state (Cubbage et al., 1997) and has been used in the Spokane River (Batts and Johnson, 1994; Bortelson et al., 1994). A 28-day test incorporating a growth end-point is proposed here to increase the sensitivity of the test. The Microtox® porewater and *Chironomus* bioassays are newly developed, but are beginning to be used at Ecology's contaminated sediment sites (e.g., ThermoRetec Consulting Corp., 2000).

The sampling, quality assurance, and analytical methods selected for the chemical analyses are consistent with those used in other sediment sampling efforts on the Spokane River (e.g., Johnson, 2000). The Horowitz 1998 metals data are an exception in that they were obtained using a total digestion procedure rather than the strong acid, partial digestion procedure used by Ecology. Although the methods differ, an interlaboratory comparison using Spokane River sediment has shown the results are comparable (Johnson, 1999).

Sampling Methods

Sampling methods will be consistent with PSEP protocols (EPA, 1996) and requirements of the Sediment Management Standards (Ecology, 1995a,b).

The samples from Sites 2, 3, 6, 7, 8, and 9 will be collected from an Ecology vessel using a 0.1 m^2 stainless steel van Veen grab. The Site 1, 4, and 5 samples will be collected by hand with a stainless steel pipe dredge or stainless steel scoops. Sampling sites will be located and positions recorded using GPS and landmarks. A grab will be considered acceptable if not over-filled with sediment, overlying water is present and not excessively turbid, the sediment surface is relatively flat, and desired depth penetration has been achieved. A field log will be maintained during sampling.

All samples will be composites of the top 10 cm layer (Ecology, 1995; EPA, 2000a). After siphoning off overlying water, the top 10 cm of sediment from each of three-to-five grabs per sampling site (seven or more if sampled with pipe dredge or scoops) will be removed with stainless steel scoops, placed in a stainless steel bowl, and homogenized by stirring. Material touching the side walls of the grab will not be taken. The samples for the Microtox test will be taken with minimum disturbance of the sediment, not homogenized, and the sample containers filled completely (no headspace) to minimize changes in pore water chemistry.

Subsamples of the homogenized sediment will be placed in glass jars with Teflon lid liners, cleaned to EPA QA/QC specifications (EPA, 1990). Sample containers, preservation, and holding times are shown in Table 2.

Stainless steel implements used to collect and manipulate the sediments will be cleaned by washing with Liquinox detergent, followed by sequential rinses with tap water, dilute nitric acid, deionized water, and pesticide-grade acetone. The equipment will then be air-dried and wrapped in aluminum foil. Between-sample cleaning of the van Veen grab will consist of thorough brushing with on-site water.

Sediment samples will be placed on ice immediately after collection and transported to Manchester Laboratory within one-to-two days. Chain-of-custody will be maintained. Manchester will ship the bioassay samples to the contract laboratories.

Back-up sampling equipment, sample containers, positioning instruments, and spare parts will be carried during field sampling as preventative maintenance.

Table 2. Sample Containers, Preservation, and Holding Times

Analysis	Container	Preservation	Holding Time
Bioassays:			
Microtox	1-liter glass; TFE-lined lid	4° C in the dark	14 days
Hyalella	1-liter glass; TFE-lined lid[a]	"	14 days
Chironomus	1-liter glass; TFE-lined lid[a]	"	14 days
Chemistry:			
Metals	8-oz. glass; TFE-lined lid	"	6 months
Organics	8-oz. glass; TFE-lined lid	"	7/14 days[b]
TOC	8-oz. glass; TFE-lined lid	"	7/14 days
Grain size	8-oz. glass; TFE-lined lid	"	6 months
% Solids	8-oz. glass; TFE-lined lid	"	7 days

[a]A total of 3 liters will be provided for the *Hyalella* and *Chironomus* tests.

Analytical Methods

Table 3 shows the analytical methods to be used, required reporting limits for the chemical analyses, and laboratories selected to conduct the work. Northwestern Aquatic Sciences, Newport, OR will do the *Hyalella* and *Chironomus* tests. Parametrix Inc., Kirkland, WA. will do the Microtox® test.

The bioassays being conducted were recently developed and no laboratories have been accredited for these specific test. Parametrix is accredited for the 1995 PSEP Microtox test. Northwester Aquatic Sciences is accredited for shorter term ASTM methods for both *Hyalella* and *Chironomus*. Both laboratories have had recent and successful experience in conducting the tests being requested of them.

In the Microtox® test, the light emitted by the bioluminescent marine bacterium *Vibrio fisheri* on exposure to test sediments is compared to a control or reference sample. The 100% porewater test is an Ecology modification (Adolphson, 2000) of Puget Sound Estuarine Protocols (PSEP) that use organic or aqueous extracts (see Appendix A).

Hyalella azteca is an amphipod. The test measures survival and growth after a 28-day exposure to test sediment. The protocol being followed is a 42-day test for survival,

growth, and reproduction (EPA Method 100.4) which is terminated before the reproduction phase EPA (2000a).

Survival and growth of the midge, *Chironomus tentans*, are the end points of this 20-day chronic test. The method is a modification of a 50-to-65 day life-cycle test (Method 100.5; EPA 2000a).

Table 3. Analytical Methods, Reporting Limits, and Laboratories

Analysis	Reporting Limit	Method	Laboratory
Bioassays:			
Microtox pore water	na	Ecology Protocol	Parametrix
Hyalella 28-day	na	Method 100.4 (EPA, 2000a)	N.W. Aquatic Sciences
Chironomus 20-day	na	Method 100.5 (EPA, 2000a)	"
Chemistry:			
Zinc	0.5 mg/Kg, dry	ICP/AES - SW6010B	Manchester
Cadmium	0.5 mg/Kg, dry	ICP/AES - SW6010B	"
Lead	5 mg/Kg, dry	GFAA - SW7421	"
Antimony	5 mg/Kg, dry	ICP/AES - SW6010B	"
Arsenic	0.2 mg/Kg, dry	GFAA - SW7060	"
Beryllium	0.1 mg/Kg, dry	ICP/AES - SW6010B	"
Chromium	1 mg/Kg, dry	ICP/AES - SW6010B	"
Copper	1 mg/Kg, dry	ICP/AES - SW6010B	"
Mercury	0.003 mg/Kg, dry	CVAA - EPA245.5	"
Nickel	1 mg/Kg, dry	ICP/AES - SW6010B	"
Selenium	0.3 mg/Kg, dry	GFAA - SW7740	"
Silver	1 mg/Kg, dry	ICP/AES - SW6010B	"
Thallium	0.2 mg/Kg, dry	GFAA - SW7841	"
Semivolatiles	5-25 ug/Kg, dry	GC/MS - SW8270	"
PCBs	5 ug/Kg, dry	GC/ECD - SW8082	"
Grain Size[a]	0.1%	Sieve & Pipet - PSEP (1996)	"
Total Organic Carbon	0.1%	Combustion/CO^2 - PSEP (1996)	"
Percent Solids	0.1%	Gravimetric - PSEP (1996)	"

[a] Gravel, sand, silt, and clay fractions

Table 4 has an estimate of laboratory costs. The number of chemistry samples shown includes a laboratory duplicate

Table 4. Cost Estimate for Analyzing Spokane River Sediment Samples

Analysis	Number of Samples*	Matrix Spikes	Total Analyses	Cost/ Analysis	Cost Subtotals
Bioassays:					
Microtox	9	na	9	450	4050
Hyalella	9	na	9	1150	10350
Chironomus	9	na	9	960	8640
					23040
		+ 25% Manchester surcharge =			5760
					28800
Chemistry:					
PP metals	10	2	12	186	2232
PCBs	10	2	12	91	1092
Semivolatlies	10	2	12	412	4944
TOC	10	0	10	33	330
Solids	10	0	10	21	210
Grain size	10	0	10	100	1000
					9808
				Total cost =	**$38,608**

Quality Control Procedures

The reference sediment for the bioassays will be Bead Lake (Site 9).

Details of the QC procedures to be followed for the sediment bioassays are described in the methods referenced in this QAPP. Eight replicates each will be used for the *Hyalella* and *Chironomus* tests. The Microtox® test uses five replicates. Frequency of water

quality monitoring, control limits, control samples, and test acceptability criteria for the bioassays are listed in Appendix B.

The QC procedures routinely followed by Manchester Laboratory for the chemical analyses requested will be satisfactory for purposes of this project. QC samples for the metals analysis will include a laboratory duplicate (split), method blank, one matrix spike, one matrix spike duplicate, and a laboratory control sample (LCS). QC samples for organics analyses will include a laboratory duplicate, method blanks, one matrix spike, and one matrix spike duplicate for each parameter, and surrogate compounds added to each sample prior to extraction. The project lead will identify the sample to be used for the duplicate and matrix spikes. QC samples for TOC, grain size, and percent solids will include a laboratory duplicate and a LCS (TOC only). QC samples, frequency, and control limits are listed in Appendix C.

Data Assessment Procedures and Reporting

The laboratories reporting of the bioassay results must include the EPA (2000a) requirements listed in Appendix D. Their statistical analysis of the data will include comparison to both the laboratory negative control and the reference sediment (Site 9), using a t-test at a significance level of 0.05.

Manchester's SOP for reduction, review, and reporting of the chemical data will meet the needs of this project. Each laboratory unit assembles data packages consisting of raw data from the analyses of the samples, copies of the pertinent logbook sheets, QA/QC data, and final reports of data entered into LIMS. These data packages are subjected to a data verification and quality assurance review by another analyst familiar with the procedure. Reviewers use *US EPA Contract Laboratory Program National Functional Guidelines for Inorganic Data Review* February, 1994 and *USEPA Contract Laboratory Program National Functional Guidelines for Organic Data Review*, October, 1999.

On receipt of the bioassay and chemical data, the project lead will review the results for completeness, reasonableness, and usability. The bioassay data will be reviewed to assure that the methods and test conditions were followed and that results on negative controls and reference toxicants were acceptable. The chemical data and case narratives will be reviewed to assure that quality control procedures met frequency requirements and control limits. After data review is completed and following any corrective actions required, the complete biological and chemical data will be forwarded to the clients.

The project lead will provide a draft report of the study results to the clients in March 2001. The report will contain:

- a map of the study area showing sampling sites
- latitude/longitude and other location information for each sampling site

- descriptions of field and laboratory methods
- a discussion of data quality and the significance of any problems encountered in the analyses
- summary tables of the biological and chemical data
- an evaluation of significant findings
- recommendations for follow-up work if warranted.

A final report will be prepared after receiving review comments from TCP and internal comments from EAP. The goal is to have the revised final report completed in May 2001. The data will be entered into Ecology's Environmental Information Management (EIM) system and Sediment Quality Information System (SEDQUAL).

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APPENDIX A. Department of Ecology Microtox Porewater Protocol

(Draft Final 8/15/00, Peter Adolphson)

Microtox® 100 Percent Sediment Porewater Toxicity Assessment

Background:
Microtox is a rapid method of assessing toxicity in aqueous media by utilizing the bioluminescent properties of the marine bacteria *Vibrio fisheri.* The test method assumes that light emitted by the bacteria can be used as an accurate assessment of the overall biological condition of the bacteria exposed to chemical compounds and mixtures. Light emitted by the bacteria exposed to potentially toxic samples is compared to light emitted to unexposed bacterial controls. Differences in luminescence are therefore deemed an indication of relative toxicity.

EPA has recommended Microtox for TIE/TRE applications (EPA/600/2-88/070) as well as stormwater investigations. Successful applications also include NPDES compliance and sediment evaluations in freshwater, estuarine and marine applications. Washington State PSEP (Puget Sound Estuarine Protocols) uses both an organic and an aqueous extraction protocol to assess sediment toxicity.

Recognizing that the goal of most sediment toxicity studies is to determine if ecologically/toxicologically significant differences exist between reference and investigative site sediments, four significant differences exist between the PSEP protocol and this revised protocol. 1) Extraction procedures are 100% pore water extraction rather than complex organic and aqueous extractions; 2) No serial dilutions are performed because LC50 calculations are not required to assess sediment toxicity between reference and site sediments; 3) No MOAS (Microtox Osmotic Adjusting Solution) is utilized; and 4) Statistical procedures utilize standard Analysis of Variance (ANOVA) or t-test procedures.

Microtox Test Procedure:

Porewater Extraction and Adjustment:
The general Microtox procedure involves centrifugation of 500ml of both reference and test sediments at approximately 4500G in for 30 minutes resulting in approximately 50 ml of pore water. Approximately 25mls of pore water is then pipetted into a clean glass container. The remaining porewater volume is set aside if needed for reducing salinity should the initial salinity adjustments steps outlined below result in the sample exceeding 22ppt.

The sample is then adjusted for salinity, dissolved oxygen and pH in the following order.

1) Salinity is adjusted to 20± 2ppt using commercially available dry bulk marine aquarium reef salts (e.g. Forty Fathoms Reef®). **[Note: The salinity adjustment**

step is omitted for Marine and estuarine sediments whose porewater exceeds 20ppt salinity.]

2) The dissolved oxygen (DO) is then adjusted by gentle aeration or agitation until it is between 50-100% saturation.
3) The pH of the salinity and DO adjusted reference and test sediment pore water should not differ from each other by more than 0.4 pH units. The pH is adjusted to 7.9-8.2 (if necessary) using a micropipette and a dilute solution (0.5 N) NaOH or HCl. Total volume of NaOH and/or HCl should be recorded. Final concentration [compared with 100% porewater extracted] can then be calculated using these data. Final dilution should not be reduced below 90% of the pore water extract. **[Note: The control solution is prepared by using deionized or distilled water and adjusting salinity, DO and pH as described above.]**

Preparation of Bacterial Suspension and Bioassay Test Setup:

A vial of freeze-dried bacteria is rehydrated with 1.0 ml of Microtox® Reconstitution solution and allowed to equilibrate for 30-90 minutes in the 4-degree Microtox Analyzer well. ***[NOTE: Mixing of the reconstituted bacteria is essential. Mix the reconstituted solution with a 1 ml pipette a minimum of 20 times by pipetting. First pipette the solution from the bottom of the cuvette and deposit the pipetted solution on the surface of the liquid remaining in the cuvette. Then pipette 1 ml of solution from the bottom of the cuvette and slowly pipette the liquid into the bottom of the cuvette.]***

One (1.0) ml of control solution is then placed in each of 5 test cuvettes and placed into the 15-degree incubation chambers. This procedure is followed for the laboratory control solution, reference sediment porewater samples, and test sediment porewater samples, for up to 4 test sediments/batch (5 pseudo-replicates per site).

In each of the test, reference, and control sample cuvettes, 10 uL of rehydrated bacteria suspension is added at 30 second intervals, immediately mixed using a 1ml pipette and allowed to incubate for 5 minutes. Used pipette tips are replaced with clean tips after each series of 5 pseudo-replicates (ref, control, and each test series ex: A1-A5). ***[NOTE: Extreme care must be used when pipetting these low volumes as slight residual amounts or presence of air bubbles in the pipette may cause variation due to error by as much as 100%.]***

Data collection:

At the initial (I_0) 5 minute mark, the first control vial is placed into the read chamber to "set" the instrument. At 30-second intervals each cuvette (inclusive of A1) is placed into the read chamber for the initial reading (I_0). After 5 additional minutes a second reading (I_5) is obtained following the above procedure. A 15-minute (I_{15}) is obtained in an additional 10 minutes.

Data analysis:

Statistical calculations are performed using a standard t-test by comparing reference with test site data. No gamma correction is required. Statistically significant differences with

$\alpha = 0.05$ and the following relative differences are indications of test failure. Relative differences between reference and test results of $\geq$15% indicate SQS failure. Relative differences between reference and test results of $\geq$25% indicate CSL failure. Relative differences are calculated as follows:

RD = [100-(T/R*100)]

RD: Relative difference
T: Mean (5 pseudo-replicates) Test output results
R: Mean (5 pseudo-replicates) Reference test output results

Control output should exceed 80 percent at the 5 minute reading and 65% at the 15 minute reading.

APPENDIX B. QC Requirements for Sediment Bioassay (modified from Ecology, 1995)

TABLE B1. SEDIMENT TOXICITY TEST CONDITIONS

Toxicity Test *Test Species*	Frequency of Water Quality Monitoring		Control Limits			Control Samples		Test Acceptability
	Hardness, alkalinity, conductivity, pH, ammonia	Temp., D.O.	Temp (°C)	Dissolved Oxygen (% sat.)	Negative Control	Positive Control	Reference Sediment	
Amphipod *Hyalella azteca*	Beginning and end of test	Daily	23±1	>40%	Clean sediment	Reference toxicant Cadmium	Yes	Mean survival in control sediment ≥80 percent. Mean weight of surviving controls≥ 0.1 mg
Midge *Chironomus tentans*	Beginning and end of test	Daily	23±1	>40%	Clean sediment	Reference toxicant Cadmium	Yes	Mean control survival ≥ 70% and minimum weight of survivors 0.6 mg
Microtox® (100% pore water) *Vibrio fisheri*	NA	NA	15	NA	Control solution	Reference toxicant Cadmium	Yes	Control output > 80% @ 5 minutes and >65% @ 15 minutes

APPENDIX C. QC Requirements for Physical/Chemical Analysis of Sediment Samples (modified from Ecology, 1995)

TABLE C1. QUALITY CONTROL PROCEDURES FOR ORGANIC ANALYSES

Quality Control Procedure	Frequency	Control Limit	Corrective Action
Instrument Quality Assurance/Quality Control			
Initial Calibration	As recommended by PSEP (1989a) and specified in analytical protocol	≤30 %RSD for SVOCs; ≤20 %RSD for PCBs Relative response factors ≥0.05 for SVOCs	Laboratory to recalibrate and reanalyze affected samples or qualify results
Continuing Calibration	After every 10-12 samples or every 12 hours, whichever is more frequent, and after the last sample of each work shift	≤25 %D for SVOCs ; ≤15 %RPD for PCBs Relative response factors ≥ 0.05 for SVOCs	Laboratory to recalibrate and reanalyze affected samples or qualify results

Method Quality Assurance/Quality Control			
Holding Times	Not applicable	1 year (samples stored frozen [-18°C]) or 14 days (samples stored at 4°C) for SVOCs and PCBs; analyze extract within 40 days	Qualify data or collect fresh samples
Method Blank	With every extraction batch	Analyte concentration $\leq$ PQL (the LOD constitutes the warning limit)	Laboratory to eliminate or greatly reduce contamination; reanalyze affected samples. If problem persists, qualify results on affected samples.
Surrogate Compounds	Added to every sample as specified in analytical protocol	EPA CLP control limits	Laboratory to follow EPA CLP protocols (reanalyzes or reextraction may be required)
Matrix Spike Sample and Matrix Spike Duplicate	With every sample batch or every 20 samples, whichever is more frequent	Recovery of 50–150 percent; precision of ≤50 RPD	Follow EPA CLP protocols
Internal Standards	Added to every sample as specified in analytical protocol	Area response of 50–200 percent of calibration standard; retention time within 30 seconds of calibration standard	Laboratory to correct problem and reanalyze affected samples. If problem persists, qualify results on affected samples.
Duplicate Sample Analysis	With every sample batch or every 20 samples, whichever is more frequent	+/-35 RPD	Laboratory may be able to correct or minimize the problem, or qualify and accept data as reported.
Detection Limits	Not applicable	(see Table 3)	Laboratory must initiate corrective actions (which may include additional cleanup steps as well as other measures) and contact the project leader immediately.

TABLE C2. QUALITY CONTROL PROCEDURES FOR METAL ANALYSES

Quality Control Procedure	*Frequency*	*Control Limit*	*Corrective Action*
Instrument Quality Assurance/Quality Control			
Initial Calibration	Daily	Correlation coefficient ≥0.995	Laboratory to recalibrate the instrument and reanalyze any affected samples
Initial Calibration Verification	Immediately after initial calibration	90–110 percent recovery (80–120 percent for mercury)	Laboratory to resolve discrepancy prior to sample analysis
Continuing Calibration Verification	After every 10 samples or every 2 hours, whichever is more frequent, and after the last sample	90–110 percent recovery (80–120 percent for mercury)	Laboratory to recalibrate and reanalyze affected samples
Initial and Continuing	Immediately after initial	Analyte concentration $\leq$ Table	Laboratory to recalibrate and

Quality Control Procedure	Frequency	Control Limit	Corrective Action
Calibration Blanks	calibration, then 10 percent of samples or every 2 hours, whichever is more frequent, and after the last sample	3	reanalyze affected samples
Method Quality Assurance/Quality Control			
Holding Times	Not applicable	6 months if samples are held at 4°C; 2 years if samples are frozen (-18°C); 28 days for mercury regardless of whether samples are held at 4°C or frozen	Qualify data or collect fresh samples
Method Blanks	With every sample batch or every 20 samples, whichever is more frequent	Analyte concentration $\leq$Table 3	Laboratory to redigest and reanalyze samples with analyte concentrations ≤10 times the highest method blank, or qualify results of affected samples
Laboratory Control Sample	With every sample batch or every 20 samples, whichever is more frequent	Varies with laboratory control sample	Laboratory to correct problem and redigest and reanalyze affected samples, or qualify results of affected samples
Matrix Quality Assurance/Quality Control			
Matrix Spike Sample	With every sample batch or every 20 samples, whichever is more frequent	75–125 percent recovery	Laboratory may be able to correct or minimize problem; or qualify and accept data
Duplicate Sample Analysis	With every sample batch or every 20 samples, whichever is more frequent	+/-35 RPD	Laboratory may be able to correct or minimize the problem, or qualify and accept data as reported.
Detection Limits	Not applicable	(see Table 3)	Laboratory must initiate corrective actions and contact the project leader immediately

TABLE C3. QUALITY CONTROL PROCEDURES FOR CONVENTIONAL ANALYSES

	Suggested Control Limit						
Analyte	Initial Calibration	Continuing Calibration	Calibration Blanks	Laboratory Control Samples	Matrix Spikes	Laboratory Triplicates	Method Blank
Grain size	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	35 percent RSD	NA

Total organic carbon	Correlation coefficient ≥0.995	80–120 percent recovery	Analyte concentration ≤CRDL	80–120 percent recovery	75–125 percent recovery	35 percent RSD	Analyte concentration < Table 3

APPENDIX D. Reporting Requirements for Sediment Bioassays (from EPA, 2000b)

16.4 Reporting

16.4.1 The record of the results of an acceptable sediment test should include the following information either directly or by referencing available documents:

16.4.1.1 Name of test and investigator(s), name and location of laboratory, and dates of start and end of test.

16.4.1.2 Source of control or test sediment, and method for collection, handling, shipping, storage and disposal of sediment.

16.4.1.3 Source of test material, lot number if applicable, composition (identities and concentrations of major ingredients and impurities if known), known chemical and physical properties, and the identity and concentration(s) of any solvent used.

16.4.1.4 Source and characteristics of overlying water, description of any pretreatment, and results of any demonstration of the ability of an organism to survive or grow in the water.

16.4.1.5 Source, history, and age of test organisms; source, history, and age of brood stock, culture procedures; and source and date of collection of the test organisms, scientific name, name of person who identified the organisms and the taxonomic key used, age or life stage, means and ranges of weight or length, observed diseases or unusual appearance, treatments used, and holding procedures.

16.4.1.6 Source and composition of food; concentrations of test material and other contaminants; procedure used to prepare food; and feeding methods, frequency and ration.

16.4.1.7 Description of the experimental design and test chambers, the depth and volume of sediment and overlying water in the chambers, lighting, number of test chambers and number of test organisms/treatment, date and time test starts and ends, temperature measurements, dissolved oxygen concentration (μg/L) and any aeration used before starting a test and during the conduct of a test.

16.4.1.8 Methods used for physical and chemical characterization of sediment.

16.4.1.9 Definition(s) of the effects used to calculate LC50 or EC50s, biological endpoints for tests, and a

summary of general observations of other effects.
16.4.1.10 A table of the biological data for each test chamber for each treatment, including the control(s), in sufficient detail to allow independent statistical analysis.
16.4.1.11 Methods used for statistical analyses of data.
16.4.1.12 Summary of general observations on other effects or symptoms.
16.4.1.13 Anything unusual about the test, any deviation from these procedures, and any other relevant information.
16.4.2 Published reports should contain enough information to clearly identify the methodology used and the quality of the results.